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APPLICATION NUMBER: *60/492,402*
FILING DATE: *August 04, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/24611*

Certified by

Jon W Dudas

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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☐ *Additional inventors are being named on th____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

A NOVEL PHARMACOLOGICAL PATHWAY DESTABILIZES LYSOSOMES AND TARGETS ONCOGENIC OR ABERRANT PROTEINS FOR DESTRUCTION

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* **4**
☐ Drawing(s) *Number of Sheets*
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
☒ Other (specify) **Return Postcard**

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

☒ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees
☒ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number **50-1619**
☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($) **$80.00**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.
☒ Yes, the name of the U.S. Government agency and the Government contract number are: **NIH RO1-CA72275**

Respectfully submitted,

SIGNATURE *Jane Massey Licata*

Date **8/4/03**

REGISTRATION NO. *(if appropriate)* **32,257**

Docket Number: **DC-0235**

TYPED or PRINTED NAME **Jane Massey Licata**

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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1) Provisional Patent Application Transmittal Letter (Small Entity)(2 copies);

2) Application consisting of 4 pages of Specification, including one (1) page of Claims;

3) Return Post Card; and

4) Authorization to charge deposit account in the amount of $80.00.

Jane Massey Licata
JANE MASSEY LICATA

A Novel Pharmacological Pathway Destabilizes Lysosomes and Targets Oncogenic or Aberrant Proteins for Destruction

This invention was supported in part by funds from the U.S. government (NIH Grant No. RO1-CA62275) and the U.S. government may therefore have certain rights in the invention.

This provisional patent application describes a novel pharmacological pathway that destabilizes lysosomes and targets oncogenic or other aberrant proteins for destruction. This previously unrecognized mechanism was uncovered in acute promyelocytic leukemia (APL) cells but is also active in other cells. APL cases most often result from the t(15;17) balanced chromosomal translocation, which expresses the fusion product, *Pml/Rar alpha*. *Pml/Rar alpha* can block differentiation of promyelocytes. Expression of *Pml/Rar alpha* leads to accumulation in affected cases of immature promyelocytes in the bone marrow and peripheral blood. APL cases are typically sensitive to all-*trans* retinoic acid (RA) treatment, which causes degradation of *Pml/Rar alpha* overcoming the dominant-negative effects of this translocation product. This degradation after RA-treatment occurs through proteasome as well as caspase dependent pathways. RA in turn triggers differentiation of the leukemic promyelocytes. However, RA resistant APL cases can occur and the trivalent form of arsenic (As^{+3}) has been used successfully in the treatment of these cases. Arsenic also induces degradation of *Pml/Rar alpha*, but mechanisms engaged in this degradation have not been well

understood and are distinct from those activated by RA-treatment. The findings reported here indicate that arsenic can act through a previously unknown mechanism that targets *Pml/Rar alpha* for degradation by rapidly destabilizing lysosomes. Destabilization leads to release of lysosomal enzymes, which triggers *Pml/Rar alpha* degradation in NB4 APL cells. Arsenic was able to destabilize rapidly lysosomes in these leukemic cells. This occurred within 2 hours of arsenic treatment as assessed in NB4 APL cells using a lysosome-specific targeting dye and confocal microscopy. Using SDS-PAGE and immunoblot analyses, lysosomal protein esterase was detected in the cytosolic fraction of cultured APL cells as soon as 5 minutes after arsenic treatment and at dosages that are clinically achievable. Furthermore, lysosomal cathepsin B appeared in the cytosol within 30 minutes of arsenic treatment of APL cells while cytochrome c (an activator of caspase-induced apoptosis) was not detected in the cytosol until 96 hours. Notably, proteasome or caspase inhibitors did not prevent this rapid arsenic-induced *Pml/Rar alpha* degradation, indicating that the mechanism engaged was novel. Consistent with this view, isolated lysosomes from NB4 APL cells could reproduce this degradation *in vitro*. This arsenic activated degradation program was also engaged by non-leukemic cells and was able to destroy leukemogenic and other aberrant proteins since the cystic fibrosis protein (CFTR) was also sensitive to this degradation. We conclude that this lysosome-dependent degradative pathway is novel. It was initially found in leukemic and non-leukemic cells following arsenic treatment. This therapeutic pathway can target *Pml/Rar alpha* and other aberrant proteins for destruction.

Furthermore, this activated lysosome-degradation pathway can also be used to screen for small molecule activators. This screening would identify pharmacological agents in addition to arsenic that would have therapeutic activity via induced destabilization of lysosomes that would in turn eliminate oncogenic or other aberrant proteins by triggering their destruction. We propose that this new lysosomal degradation pathway that is activated by arsenic or other compounds to be uncovered using this novel screen will have therapeutic activity. This will occur in clinical settings where the disease state depends on expression of oncogenic or aberrant proteins susceptible to this degradation.

What is claimed is:

1. A method for identifying an agent which destabilizes lysosomes to increase oncogenic or aberrant protein degradation comprising contacting a lysosome or a cell containing a lysosome with an agent and detecting whether said agent destabilizes lysosomes thereby increasing oncogenic or aberrant protein degradation.

2. An agent identified by the method of claim 1.

3. The method of claim 2, wherein the agent is arsenic.

4. A method for destabilizing lysosomes comprising administering to a lysosome or a cell containing a lysosome an agent of claim 2 so that the lysosome is destabilized.

5. The method of claim 4, wherein the lysosome is destabilized and an oncogenic or aberrant protein is degraded.

6. A method for treating a disease or condition associated with an oncogenic or aberrant protein comprising administering an agent of claim 2.

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